Academic Emergency Medicine
Official Journal of the Society for Academic Emergency Medicine

ORIGINAL CONTRIBUTION

A Prospective, Randomized Trial of Intravenous Hydroxocobalamin Versus Whole Blood Transfusion Compared to No Treatment for Class III Hemorrhagic Shock Resuscitation in a Prehospital Swine Model

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Abstract

Objectives: The objective was to compare systolic blood pressure (sBP) over time in swine that have had 30% of their blood volume removed (Class III shock) and treated with intravenous (IV) whole blood or IV hydroxocobalamin, compared to nontreated control animals.

Methods: Thirty swine (45 to 55 kg) were anesthetized, intubated, and instrumented with continuous femoral and pulmonary artery pressure monitoring. Animals were hemorrhaged a total of 20 mL/kg over a 20-minute period. Five minutes after hemorrhage, animals were randomly assigned to receive 150 mg/kg IV hydroxocobalamin solubilized in 180 mL of saline, 500 mL of whole blood, or no treatment. Animals were monitored for 60 minutes thereafter. A sample size of 10 animals per group was determined based on a power of 80% and an alpha of 0.05 to detect an effect size of at least a 0.25 difference (>1 standard deviation) in mean sBP between groups. sBP values were analyzed using repeated-measures analysis of variance (RANOVA). Secondary outcome data were analyzed using repeated-measures multivariate analysis of variance (RMANOVA).

Results: There were no significant differences between hemodynamic parameters of IV hydroxocobalamin versus whole blood versus control group at baseline (MANOVA; Wilks' lambda; $p = 0.868$) or immediately posthemorrhage (mean sBP = 47 mm Hg vs. 41 mm Hg vs. 37 mm Hg; mean arterial pressure = 39 mm Hg vs. 28 mm Hg vs. 34 mm Hg; mean serum lactate = 1.2 mmol/L vs. 1.4 mmol/L vs. 1.4 mmol/L; MANOVA; Wilks' lambda; $p = 0.348$). The outcome RANOVA model detected a significant difference by time between groups ($p < 0.001$). Specifically, 10 minutes after treatment, treated animals showed a significant increase in mean sBP compared to nontreated animals (mean sBP = 76.3 mm Hg vs. 85.7 mm Hg vs. 51.1 mm Hg; $p < 0.001$). RMANOVA modeling of the secondary data detected a significant difference in mean arterial pressure, heart rate, and serum lactate ($p < 0.001$). Similar to sBP, 10 minutes after treatment, treated animals showed a significant increase in mean arterial pressure compared to nontreated animals (mean arterial pressure = 67.7 mm Hg vs. 61.4 mm Hg vs. 40.5 mm Hg). By 10 minutes, mean heart rate was significantly slower in treated animals compared to nontreated animals (mean heart rate = 97.3 beats/min vs. 95.2 beats/min vs. 129.5 beats/min; $p < 0.05$). Serum lactate, an early predictor of shock, continued to rise in the control group, whereas it did not in treated animals. Thirty minutes after treatment, serum lactate values of treated animals were significantly lower compared to nontreated animals ($p < 0.05$). This trend continued throughout the 60-minute observation period such that 60-minute values for lactate were 1.4 mmol/L versus 1.1 mmol/L versus 3.8 mmol/L. IV hydroxocobalamin produced a statistically significant increase in systemic vascular resistance compared to control, but not whole blood, with a concomitant decrease in cardiac output.

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Received June 5, 2014; revisions received September 3 and October 7, 2014;; accepted October 29, 2014.

These data were presented at the Society for Academic Emergency Medicine Annual Meeting, Dallas, TX, May 2014.

The U.S. Air Force Office of the Surgeon General funded this study. No other funding was used. The views expressed in this article are those of the authors and do not reflect the official policy or position of the Department of the U.S. Air Force, Department of Defense, or the U.S. government. The author have no potential conflicts of interest to report.

Supervising Editor: Brian O'Neil, MD.

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doi: 10.1111/acem.12605
ISSN 1069-6563
PII ISSN 1069-6563583

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01 MAR 2015	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
A Prospective, Randomized Trial of Intravenous Hydroxocobalamin Versus Whole Blood Transfusion Compared to No Treatment for Class III Hemorrhagic Shock Resuscitation in a Prehospital Swine Model	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
Bebarta V. S., Garrett N., Boudreau S., Castaneda M.,	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	8. PERFORMING ORGANIZATION REPORT NUMBER
United States Army Institute of Surgica; Research, JBSA Fort Sam Houston, TX 78234	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**SAR**	**10**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

Conclusions: Intravenous hydroxocobalamin was more effective than no treatment and as effective as whole blood transfusion, in reversing hypotension and inhibiting rises in serum lactate in this prehospital, controlled, Class III swine hemorrhage model.

ACADEMIC EMERGENCY MEDICINE 2015;22:321–330 © 2015 by the Society for Academic Emergency Medicine

Under controlled surgical conditions, whole blood provides the essential components for resuscitation of traumatic hemorrhage and combat wounds. The recommended ratio of red blood cells, fresh-frozen plasma, and platelets approaches the reconstitution of whole blood and has been found to be effective in treating hemorrhagic shock and preventing acute organ hypoxia.[1–3] However, administration of whole blood or a combination of blood components is impractical in the prehospital setting. Furthermore, in the prehospital setting, under conditions of uncontrolled hemorrhage, limiting resuscitation fluid volumes reduces the risk of coagulopathies, a hyperinflammatory response, and exsanguination.[4–8] While whole blood transfusion is the preferred resuscitation fluid for massive hemorrhage, it is not available under austere conditions. Intravenous (IV) hydroxocobalamin may provide time for appropriate blood type and screening and limit total transfusion requirements when administered in the prehospital or emergency department because of its long half-life.[9]

Hydroxocobalamin was approved by the United States Food and Drug Administration (FDA) for treatment of cyanide toxicity after efficacy was documented both in animal studies and in human case series in Europe.[10] Hydroxocobalamin has been shown to effectively increase blood pressure in cyanide-induced cardiac arrest and endotoxin-induced hypotension.[11–13] In previous studies examining the efficacy of hydroxocobalamin to treat cyanide toxicity in a swine model of acute severe cyanide toxicity, it also improved blood pressure, heart rate, and lactate levels.[14,15] Hydroxocobalamin has been shown to improve blood pressure likely through nitric oxide scavenging, when administered in a small volume, and may be an ideal resuscitation treatment prior to the availability of blood.[16] Severe hemorrhage has been associated with high nitric oxide states, and nitric oxide scavenging has been shown to be beneficial.[17] Because a small volume of hydroxocobalamin (90–180 mL) improves cardiovascular parameters, we speculated that it may be a viable treatment option for hemorrhagic shock prior to the availability of blood.

To our knowledge, there are no published reports describing the efficacy of IV hydroxocobalamin in a hypotensive, hemorrhagic, clinically relevant animal model. Moreover, preliminary results from our proof-of-concept study revealed that hydroxocobalamin was effective in increasing blood pressure as well as systemic vascular resistance in a hypovolemic hemorrhagic swine model.[18]

The primary hypothesis of this study is that hydroxocobalamin will improve systolic blood pressure (sBP) compared to control after hemorrhage induced hypotension, hence reversing the hypotension associated with hemorrhage in our swine model. Specifically, we compared sBP over time in animals that had 30% of their blood volume removed (Class III hemorrhage) and were subsequently treated with IV hydroxocobalamin or IV whole blood (positive control) compared to non-treated (negative control) animals. We selected sBP as the primary outcome because that is the cardiovascular parameter most accessible to first responders and health care providers who work under austere conditions.[19,20] However, we included other factors in our data collection and analysis that may add value in assessing the efficacy of hydroxocobalamin in reversing hypotension associated with Class III hemorrhage.

METHODS

Study Design

This was a randomized, comparative, laboratory investigation. The study was approved by our Institutional Animal Care and Use Committee at the Wilford Hall Ambulatory Surgical Center Clinical Research Division. Our study was funded by the United States Air Force Office of the Surgeon General.

Animal Handling and Preparation

All procedures involving animals complied with the regulations and guidelines of the Animal Welfare Act, the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, and the American Association for Accreditation of Laboratory Animal Care. The housing of animals and the performance of the study took place in the Animal Care Facility at our institution.

Due to the circuitous course of the urethra in male swine and the necessity to catheterize the swine for this protocol, we elected to use female swine. Thirty female Yorkshire swine (*Sus scrofa*), each weighing 45 to 55 kg, were premedicated with intramuscular ketamine 10 mg/kg. General anesthesia was induced with isoflurane via nose cone. Following endotracheal intubation, the animals were mechanically ventilated with a volume-limited, time-cycled ventilator (Fabius GS anesthesia machine, Drager-Siemens, New York, NY) and maintained with inhaled isoflurane (1%–3%) and oxygen (FiO_2 of 0.4–0.45). The tidal volume was initially 8 to 10 mL/kg and the respiratory rate was 12 breaths/min. The minute ventilation was adjusted to maintain an end-tidal carbon dioxide (CO_2) value between 38 and 42 mm Hg as measured by inline capnography. Lead II of the surface electrocardiogram was monitored continuously. Temperature was maintained at 37.5 to 39.0 °C. Baseline biochemical variables (arterial blood gas, hematocrit, and electrolytes) were measured.

Study Protocol

Invasive hemodynamic variables were measured with an eight-French Swan-Ganz CCOmbo V pulmonary artery catheter (Model 777F8) and the Edwards Vigilance II monitor (Edwards Lifesciences, Irvine, CA). Measurements included continuous cardiac output, systemic vascular resistance, mixed venous oxygen saturation, central venous pressure, pulmonary artery pressure, and core temperature. The catheter ports were flushed with saline, and the catheter was placed via cutdown in the right external jugular vein. Aortic pressure was measured continuously through the femoral artery. An 8.5 French introducer (Arrow, Reading, PA) was placed in the carotid artery for laboratory sampling and another was placed in the internal jugular vein for medication or blood administration. Each animal received a warmed saline IV bolus (10 mL/kg) during procedure setup. The Fabius GS anesthesia data collection software embedded in the ventilator's computer was used for data acquisition at 1-minute intervals.

Baseline biochemical measurements included oxygen saturation, $PaCO_2$, PaO_2, hemoglobin, pH, bicarbonate, lactate (ABL 800 Flex blood gas analyzer, Radiometer America, Westlake, OH), prothrombin time, partial thromboplastin time (STA-R Evolution, Diagnostic Stago Inc., Parsippany, NJ), and platelet count (Advia 120, Siemens, Norwood, MA).

After instrumentation, isoflurane was reduced to 1.5%, after which animals were acclimated and blood pressure was stabilized for 10 minutes before hemorrhage began. There was no significant difference in percentage of isoflurane among the groups during or after hemorrhage (mean percent isoflurane = control, 1.2; IV hydroxocobalamin, 1.5; whole blood, 1.4), and hence depth of anesthesia was not a factor in outcomes. Animals were then hemorrhaged using modified hemorrhage models from Frankel et al.[21] and White et al.[22] such that 1.42 mL/kg/min blood was removed over 7 minutes, and then 0.76 mL/kg/min blood was removed over the remaining 13 minutes. Blood was removed from the carotid arterial line via aspiration with a 60-mL syringe and saved in a blood collection system containing citrate-phosphate-dextrose with Optisol red blood cell preservative solution (Terumo Corp., Tokyo, Japan). The collected blood was then placed in a blood warmer for subsequent experimental transfusion to the same animal. Five minutes after hemorrhage, animals were randomly assigned, using a computer-generated randomization schedule,[23] to receive 150 mg/kg IV hydroxocobalamin solubilized in 180 mL of saline, 500 mL of whole blood, or no treatment. Because the focus of this study was the effectiveness of hydroxocobalamin under austere conditions, we elected to replace 500 mL of blood, rather than the full liter removed, as that is the usual amount available under austere conditions (i.e., "buddy transfusions").[24–26] The administration time of the treatments, i.e., hydroxocobalamin or whole blood, was standardized such that each was infused over a mean time period of 4 to 5 minutes. Ten milliliters of saline was infused before and after each treatment. The animals were then monitored for 60 minutes after treatment or no treatment.

Outcome Measures

The primary outcome measure was sBP over time, from hemorrhage (time 0) to 60 minutes. This outcome was defined before the study. We also compared cardiac output, mean arterial pressure, systemic vascular resistance, mixed venous oxygen saturation, and heart rate. Vital signs and hemodynamic measurements were recorded at 1-minute intervals and analyzed at 10-minute intervals. Serum blood sampling was taken at baseline; immediately after hemorrhage; and at 5, 10, 20, 30, 40, 50, and 60 minutes after treatment or no treatment.

Data Analysis

Sample size calculations were performed using G*Power Version 3.0.10 and repeated-measures analysis of variance (ANOVA) parameters. A sample size of 10 animals per group was determined based on a power of 80% and an alpha of 0.05 to detect an effect of size of at least a 0.25 difference (more than 1 standard deviation) in mean sBP between the groups. This effect size was based on previous published studies where hydroxocobalamin was used to increase sBP in a cyanide toxicity animal model.[14] We tested the sensitivity of the power to a reduction in the number of subjects in the event of mortality, which established that the number of subjects per group could be a small as four. sBP data were modeled using repeated-measures ANOVA and secondary outcome data were modeled using repeated-measures multivariate ANOVA (RMANOVA), using Type III sum-of-squares model, which accounts for unequal group sizes. As the RMANOVA was significant, six univariate repeated-measures ANOVAs were conducted with a Bonferroni adjustment for multiple testing applied ($p < 0.008$) to reduce the likelihood of a Type I error. All repeated-measures ANOVA reported were significant at the $p < 0.001$ level. Post hoc analysis was performed on all variables that showed a significant treatment by time, for which treatment contrasts were measured at each posttreatment time point with a Bonferroni adjustment for multiple testing applied. All statistical testing was two-sided and completed using SPSS version 21. Descriptive statistics were modeled using MANOVA. All graphical presentations were made using R version 2.15.1.

RESULTS

At baseline, the groups had similar vital signs and biochemical variables except for hemoglobin, which was higher in the control group (Table 1). At time 0, predefined as posthemorrhage, this significant difference persisted (Table 2). Because all of the swine were hydrated prior to the start of the experiment, all had adequate urine output, and all hemoglobin values were within the normal range for Yorkshire swine, we speculate that this difference in hemoglobin values is a statistical anomaly and not clinically relevant.

At 5 and 15 minutes after hemorrhage, two animals in the whole blood group died, leaving eight animals in the whole blood group to complete the study. Data from the nonsurviving animals were excluded from analysis. Our data analysis accounts for this circumstance. sBP,

the primary outcome variable, was similar between the hydroxocobalamin and whole blood groups over time. This was significantly different from the nontreated group such that at 10 minutes, treated animals showed a significantly greater mean sBP than to nontreated animals. Mean sBP for IV hydroxocobalamin and whole blood groups were 76.3 ± 13.0 and 85.8 ± 17.8 mm Hg, respectively, compared to 51.1 ± 10.7 mm Hg for the controls (Figure 1A). This statistically significant difference persisted over 60 minutes. Similar to sBP, heart rate (Figure 1B) and mean arterial pressure (Figure 1C) significantly improved by 10 minutes in both treated groups, but not in the control group ($p < 0.008$). At 10 minutes, the mean heart rates were 97.3 ± 26.5 and 95.2 ± 14.9 beats/min, and the mean arterial pressures were 61.4 ± 13.9 and 67.8 mm ± 17.0 mm Hg for the hydroxocobalamin and whole blood groups, whereas control animals displayed a mean heart rate of 129.5 ± 40.9 beats/min ($p < 0.05$) and mean arterial pressure of 40.5 ± 8.3 mm Hg ($p < 0.05$). These statistically significant differences were sustained. Correspondingly, serum lactate, an early predictor of hemorrhagic shock,[27] was significantly different among groups over time such that at 30 minutes after treatment there was a significant increase in serum lactate for the control animals that persisted through the 60-minute observation period. By 60 minutes, serum lactate levels for the hydroxocobalamin and whole blood groups were 1.36 ± 0.8 and 1.1 ± 0.25 mmol/L, but control animals' lactate levels were 3.8 ± 5.0 mmol/L. Consequently, IV hydroxocobalamin and whole blood groups lactate levels were not significantly different from each other, but were each significantly different from control animal lactate levels (Figure 1D). Whereas serum lactate in control animals increased 263% from baseline, hydroxocobalamin-treated animals sustained a 36% increase, and whole blood–treated animals sustained a 10% increase. Although there was a significant difference in measured lactate, serum pH values were not significantly different between the groups and were within normal limits (7.42 ± 0.03 vs. 7.41 ± 0.06 vs. 7.45 ± 0.06; 60-minute values reported), nor was there a significant difference in base excess. We note that in other studies examining biological markers of shock in ventilated subjects, serum lactate levels rise early, whereas serum pH and base excess change less in the early stages of shock.[28–30]

Animals in the hydroxocobalamin group demonstrated significantly greater systemic vascular resistance over time compared to nontreated animals, but not compared to animals treated with whole blood (mean systemic vascular resistance at 60 minutes 1316 ± 216 vs. 882 ± 270 vs. 973 ± 183 dynes-sec/cm^5; Figure 1E). In addition, mean cardiac output (3.4 ± 0.3 vs. 3.3 ± 0.27 vs. 4.6 ± 0.3 L/min) was significantly lower in the hydroxocobalamin and nontreated animals compared to whole blood–treated animals (Figure 1F). Ten-minute interval data from hemorrhage through the 60-minute observation are presented in Table 3.

Data analysis showed that coagulation markers for hydroxocobalamin versus whole blood versus control groups were not significantly different (mean 60-min prothrombin time = 14.6 ± 0.5 vs. 13.5 ± 0.3 vs. 13.6 ± 0.5 sec; partial thromboplastin time = 33.8 ± 3.9 vs. 25.5 ± 6.7 vs. 32 7.1 sec; platelet count [$\times 10^9$/L] = 329 ± 63 vs. 267 ± 60 vs. 359 ± 76).

DISCUSSION

To the best of our knowledge, this is the first investigation examining the efficacy of hydroxocobalamin in swine using 30% blood loss as the hemorrhagic shock model. We found that over the 60-minute observation period, IV hydroxocobalamin was comparable to whole blood in improving sBP, mean arterial pressure, and heart rate, compared to prehemorrhage baseline values. In fact, hydroxocobalamin and whole blood provided significant benefit with regard to those cardiovascular parameters compared to no treatment (controls), beginning at 10 minutes after treatment, and this difference was sustained over 60 minutes. Although IV hydroxocobalamin produced a significant increase in systemic vascular resistance compared to the control group, but not the whole blood group, this may be attributed to its nitric oxide scavenging properties.[13,16] Nitric oxide synthase has been shown to be upregulated in hemorrhagic shock[31] and septic shock,[32] resulting in a large release of nitric oxide, hence the scientific interest in nitric oxide scavengers or nitric

Table 1
Baseline Characteristics of the Animals 30 Minutes Before Hemorrhage

Characteristics	Whole Blood (n = 8)	Hydroxocobalamin (n = 10)	Control (n = 10)
Weight, kg	49.5 (±2.9)	48.2 (±2.6)	51.8 (±2.4)
Heart rate, beats/min	85 (±9)	92 (±16)	88 (±16)
sBP, mm Hg	104 (±11)	103 (±6)	102 (±11)
Mean arterial pressure, mm Hg	82 (±13)	81 (±7)	78 (±11)
Cardiac output, L/min	4.9 (±0.5)	5.1 (±1)	5.2 (±1)
Systemic vascular resistance, dynes-sec/cm^5	1,229 (±186)	1,186 (±267)	1,102 (±257)
Lactate, mmol/L	1.0 (±0.3)	1.0 (±0.3)	1.0 (±0.3)
Hemoglobin, g/dL*	7.7 (±0.8)	7.4 (±0.8)	8.9 (±0.5)
Prothrombin time, sec	13.6 (±0.1)	13.6 (±0.4)	13.4 (±0.4)
Partial thromboplastin time, sec	29.8 (±6.1)	28.8 (±3.7)	32.3 (±7.3)

Data are presented as means (±SD).
*Significant difference ($p < 0.05$).

Table 2
Immediate Posthemorrhage Characteristics of the Animals

Characteristics	Whole Blood (n = 8)	Hydroxocobalamin (n = 10)	Control (n = 10)
Blood loss, % of total blood volume	30 (±0.00)	30 (±0.01)	30 (±0.01)
Heart rate, beats/min	86 (±8.8)	95 (±7.9)	107 (±7.9)
sBP, mm Hg	40.9 (±3.5)	47.1 (±3.1)	36.6 (±3.1)
Mean arterial pressure, mm Hg	34.2 (±2.9)	38.5 (±2.6)	29.5 (±2.6)
Cardiac output, L/min	3.01 (±0.3)	3.5 (±0.27)	3.0 (±0.27)
Systemic vascular resistance, dynes-sec/cm^5	797.9 (±79)	790.1 (±71)	668 (±71)
Lactate, mmol/L	1.37 (±0.4)	1.21 (±0.3)	1.39 (±0.6)
Hemoglobin, g/dL*	7.4 (±0.7)	7.5 (±0.6)	8.3 (±0.5)
Prothrombin time, sec	13.5 (±0.5)	13.6 (±0.4)	13.4 (±0.3)
Partial thromboplastin time, sec	27.0 (±4.9)	27.5 (±4.2)	31.9 (±7.8)

Data are presented as means (±SD).
*Significant difference ($p < 0.05$).

oxide synthase inhibitors for the treatment of shock. Whereas experiments using nitric oxide synthase inhibitors have had mixed results because the compounds inhibit both inducible and constitutive nitric oxide synthase,[33–36] nitric oxide scavengers have shown promise.[13,37,38]

In this study, cardiac output in the IV hydroxocobalamin group was significantly lower compared to whole blood group, but not the control group. We speculate that cardiac output was decreased in the IV hydroxocobalamin group because hydroxocobalamin produced an increase in systemic vascular resistance via nitric oxide scavenging. However, in the nontreated animals, neither systemic vascular resistance nor cardiac output were significantly increased compared to the treated groups, suggesting a deterioration of cardiovascular status in that group. Furthermore, whereas serum lactate levels were near normal by 60 minutes in the treated groups, the control group was experiencing elevated lactate levels, suggesting continued shock.

Traumatic hemorrhage is a leading cause of death in civilian and military environments and is the cause of 40% of civilian deaths and 50% of military deaths.[39–41] Furthermore, a loss of 30% to 40% blood volume has been reported as the most frequent preventable cause of hemorrhagic death.[39,42] Hemorrhagic injuries to extremities are effectively treated with hemostatic bandages and tourniquets.[43,44] However, abdominal and thoracic hemorrhage is often lethal, and better resuscitation therapies are being studied because hemorrhage from these types of injuries cannot be immediately controlled.[45]

In the prehospital, tactical combat environment, early resuscitation is important; however, studies of resuscitation fluids have provided mixed benefit. Large-volume crystalloid resuscitation can dilute coagulation factors and worsen survival.[6,46,47] Current Committee on Tactical Combat Casualty Care guidelines recommend 500 mL of Hextend (Biotime Inc.), a synthetic colloid, because of the smaller weight and volume.[48] However, studies have not confirmed efficacy in shock and have documented risk including coagulopathies.[49–52] Hypertonic saline has also been studied because of the small volume and potential benefit for head trauma. However, a large NIH-sponsored trial did not demonstrate clear benefit.[53] Hemoglobin-based oxygen carriers are attractive candidates, but the results of studies of these carriers have been limited by clinical side effects, lack of efficacy, and increased deaths.[54–57] Finally, IV plasma may be effective for shock; however, it is difficult to deliver and store in prehospital, tactical settings, and lyophilized or freeze-dried plasma has not been fully studied.[58–60] In addition, plasma and other blood products are expensive and carry infection risks.[61] A small-volume, simple, durable, portable drug that improves blood pressure and survival is needed to treat hemorrhagic shock in the tactical environment, and hydroxocobalamin may be a potential candidate based on our study results.

Hydroxocobalamin is safe and FDA-approved for cyanide toxicity and has been shown to effectively increase blood pressure in cyanide-induced cardiac arrest and endotoxin-induced hypotension.[11,12,62,63] The improvement in blood pressure under these conditions is likely due to nitric oxide scavenging.[16] Animal models suggest that hydroxocobalamin scavenges nitric oxide and regulates nitric oxide and nitric oxide synthase selectively.[13,16] Hence, in our model, hydroxocobalamin increased systemic vascular resistance significantly compared to whole blood or no treatment and produced a concomitant decrease in cardiac output. This outcome was not unexpected and may be beneficial when blood products are unavailable.

Hydroxocobalamin infusion did not dilute coagulation factors because of the small volume (90–180 mL) compared to a crystalloid infusion (1,000–3,000 mL). Moreover, data analysis showed that coagulation markers for IV hydroxocobalamin versus whole blood versus control groups were not significantly different, suggesting that hydroxocobalamin does not have a direct effect on coagulation. Finally, if other causes of shock are ongoing from sepsis or chemical terrorism, hydroxocobalamin would be beneficial, thus offering one safe drug for several types of combat-induced shock in a tactical environment.

In this study, comparing the efficacy of hydroxocobalamin to no treatment to mitigate acute hemorrhage compared to whole blood transfusion, we found that hydroxocobalamin was significantly better than control in improving sBP and provided a similar benefit to

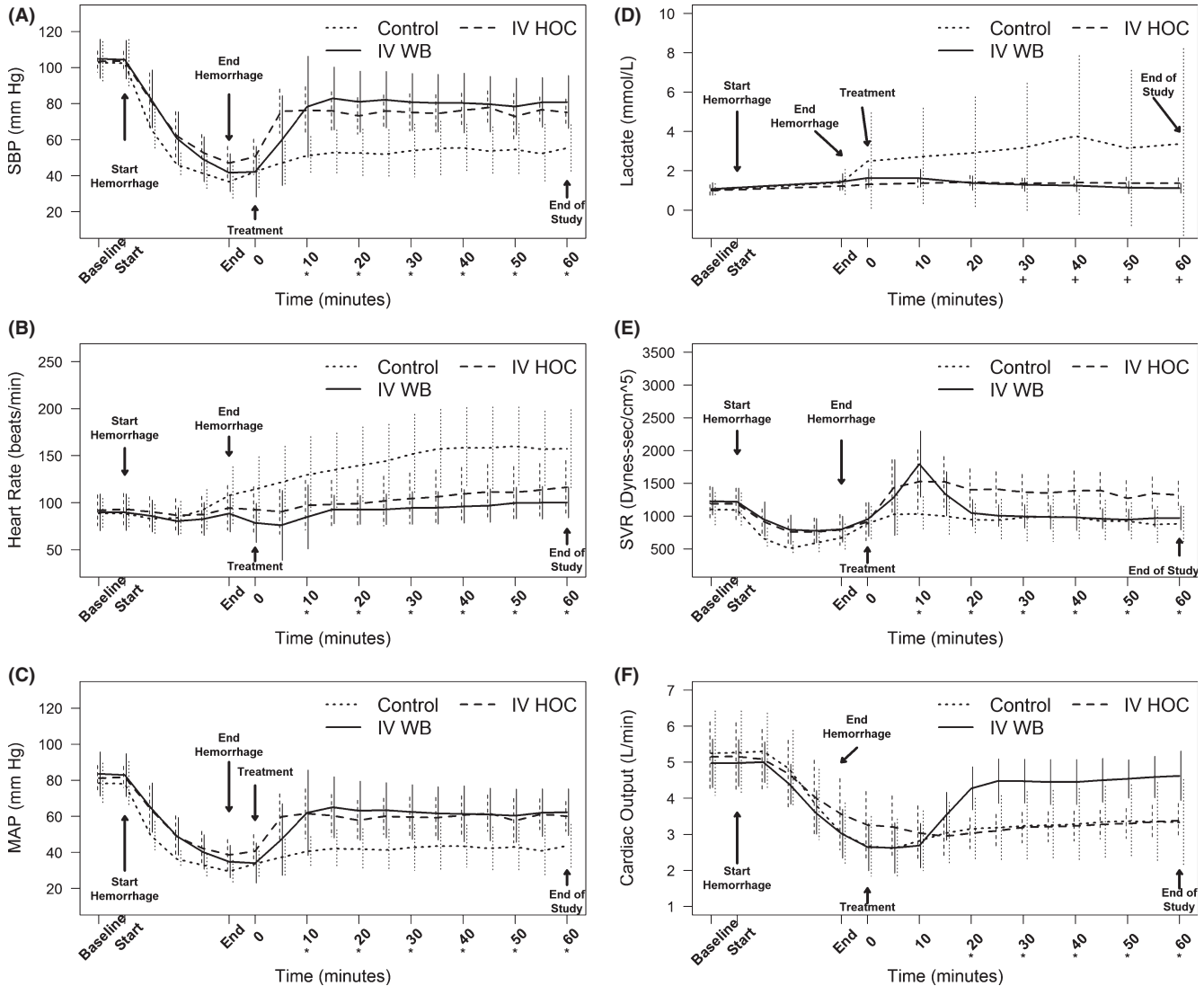

Figure 1. Hemodynamic variables (sBP, MAP, SVR, heart rate, and cardiac output) and serum lactate measured in animals over time until the end of the experiment. Animals were hemorrhaged such that 30% of the total blood volume was removed over 20 minutes. Five minutes after hemorrhage animals were treated with IV HOC (dashed line; $n = 10$), IV WB (solid line; $n = 8$), or not treated (control; dotted line; $n = 10$) and observed for 60 minutes. All repeated-measures ANOVAs were significant at $p < 0.001$. Treatment by time are designated with *$p < 0.001$ or †$p < 0.03$. (**A**) sBP measured in animals over time through 60 minutes after treatment; (**B**) heart rate measured in animals over time over time through 60 minutes after treatment; (**C**) MAP measured in animals over time through 60 minutes after treatment; (**D**) serum lactate measured in animals over time through 60 minutes after treatment; (**E**) SVR measured in animals over time through 60 minutes after treatment; (**F**) Cardiac output measured in animals over time through 60 minutes after treatment. IV HOC = intravenous hydroxocobalamin; IV WB = intravenous whole blood; MAP = mean arterial pressure; SVR = systemic vascular resistance.

whole blood transfusion in treating hypotension and improving serum lactate. Similar to previous studies, blood pressure initially increased markedly after hydroxocobalamin administration, but then lowered over time, normalizing toward baseline by the end of the experiment. A longer observation period may be valuable, but was beyond the scope of our study, which focused on the initial resuscitation of Type III hemorrhagic shock.

LIMITATIONS

The primary limitation is that an animal model does not precisely reproduce human hemorrhagic shock. We chose a swine model because it has been used in previous investigations of hemorrhagic shock and because the swine cardiovascular system is analogous to humans.[64,65]

Second, we modified the hemorrhage models rates of hemorrhage in the studies by Frankel et al.[21] and the White et al.[22] of 2.15 mL/kg/min over 7 minutes; then 1.15 mL/kg/min over the remaining 13 minutes, such that we removed 1.42 mL/kg of blood over 7 minutes; and then 0.76 mL/kg of blood over the remaining 13 minutes. In our model, this modification provided a consistent 30% blood loss in all groups, i.e., IV hydroxocobalamin versus IV whole blood versus no treatment (mean [±SD] percentage of total

Table 3
Hemodynamic and Serum Lactate Values From Hemorrhage to 60 Minutes

	Time from Hemorrhage (Time 0) Through 60 Minutes						
Variable	0	10	20	30	40	50	60
sBP, mm Hg							
WB	44.8 (±13.2)	85.8 (±15)	81.0 (±17)	80.9 (±16)	80.3 (±17)	78.4 (±16)	80.9 (±15)
HOC	50.4 (±9.9)	76.3 (±13)	73.1 (±10)	75.1 (±10)	76.2 (±10)	72.8 (±13)	75.1 (±8)
CTRL	42.2 (±9.2)*	51.1 (±11)*	52.6 (±13)*	53.9 (±15)*	55.4 (±13)*	54.5 (±14)*	55.3 (±13)*
p-value	0.23	0.000	0.000	0.001	0.001	0.003	0.000
MAP, mm Hg							
WB	36.1 (±11)	67.8 (±17)	63.1 (±16)	62.4 (±15)	61.4 (±16)	60.4 (±15)	62.2 (±13)
HOC	40.7 (±9)	61.4 (±14)	57.8 (±11)	59.6 (±11)	60.7 (±11)	57.6 (±12)	60.0 (±8)
CTRL	33.7 (±7)	40.5 (±8)*	41.8 (±11)*	42.7 (±13)*	43.5 (±12)*	42.9 (±13)*	43.6 (±12)*
p-value	0.23	0.000	0.004	0.006	0.007	0.019	0.002
SVR,† dynes-sec/cm^5							
WB	934 (±273)	1792 (±505)	1046 (±205)	993 (±179)	981 (±200)	943 (±161)	973 (±184)
HOC	913 (±289)	1526 (±487)*	1399 (±322)*	1366 (±271)*	1387 (±303)*	1269 (±273)*	1316 (±217)*
CTRL	886 (±263)	1029 (±269)	945 (±240)	975 (±356)	979 (±335)	922 (±282)	882 (±270)
p-value	0.99	0.003	0.002	0.008	0.006	0.008	0.001
HR, beats/min							
WB	83.5 (±19)	95.2 (±15)	92.5 (±15)	94.4 (±17)	96.1 (±18)	99.6 (±17)	100.4 (±17)
HOC	92.4 (±25)	97.3 (±27)	99 (±27)	104.5 (±27)	109.4 (±28)	110.8 (±28)	116.5 (±28)
CTRL	114.2 (±35)	129.5 (±41)*	139.6 (±41)*	151.5 (±43)*	158.3 (±43)*	160.0 (±42)*	157.3 (±42)*
p-value	0.63	0.035	0.004	0.001	0.001	0.001	0.002
Cardiac output, L/min							
WB	2.7 (±0.7)	2.7 (±0.6)	4.3 (±0.6)*	4.5 (±0.6)*	4.5 (±0.6)*	4.5 (±0.5)*	4.6 (±0.7)*
HOC	3.2 (±0.9)	3.0 (±0.8)	3.0 (±0.4)	3.2 (±0.4)	3.2 (±0.4)	3.3 (±0.4)	3.4 (±0.5)
CTRL	2.7 (±0.8)	2.8 (±0.8)	3.2 (±0.9)	3.2 (±1.0)	3.3 (±1.0)	3.4 (±1.0)	3.3 (±1.1)
p-value	0.24	0.25	0.001	0.001	0.002	0.002	0.006
Lactate, mmol/L							
WB	1.6 (±0.4)	1.5 (±0.4)	1.4 (±0.4)	1.2 (±0.3)	1.3 (±0.3)	1.2 (±0.3)	1.1 (±0.3)
HOC	1.3 (±0.3)	1.4 (±0.2)	1.4 (±0.3)	1.4 (±0.3)	1.4 (±0.3)	1.4 (±0.3)	1.4 (±0.8)
CTRL	2.7 (±2.6)	2.9 (±2.4)	3.1 (±2.9)	3.4 (±3.0)*	4.0 (±4.0)*	3.6 (±4.5)*	3.8 (±5.0)*
p-value	0.13	0.07	0.07	0.048	0.036	0.05	0.05

All values are mean (±SD).
p-values represent post hoc analysis comparing the three groups using the Bonferroni correction method.
CTRL = control group; HOC = hydroxocobalamin; HR = heart rate; MAP = mean arterial pressure; SVR = systemic vascular resistance; WB = whole blood.
*Significant difference.
†Significant differences were only detected between the HOC and the CTRL group.

blood volume extracted 30% [±0.01%] vs. 30% [±0.00%] vs. 30% [±0.01%]). Different volume percentages of blood loss may have had different outcomes.

Third, observing the animals for a longer period may have shown a difference between the hydroxocobalamin and the whole blood groups. Additionally, a longer observation may have detected differences in oxygen debt measured by mixed venous oxygen saturation or other biological markers of shock that appear later.[28] Finally, our model was a volume-controlled hemorrhage model. The advantage of a controlled hemorrhage model is that the amount blood loss is consistent across experimental groups and findings from controlled hemorrhage studies are reproducible across species.[66] Furthermore the model by Frankel et al. of a rapid rate of hemorrhage followed by a slower rate of hemorrhage, the model we employed, has been suggested to be the best model of controlled-volume hemorrhage models.[67] Moreover, controlled hemorrhage studies continue to be used as valid research tools with data from the findings reported in peer-reviewed journals.[68–70] Nevertheless, we recognize that controlled hemorrhage may not precisely replicate traumatic hypotension and subsequent surgical intervention associated with traumatic injury. Hence it may be difficult to produce results consistent with ours in an uncontrolled hemorrhagic model. To provide support for results from this study, we have planned future studies in which we will use a model of uncontrolled hemorrhage, specifically a groin injury model.

CONCLUSIONS

Intravenous hydroxocobalamin was more effective than no treatment, and as effective as whole blood transfusion, for the reversal of hypotension and inhibiting rises in serum lactate in our prehospital, controlled, Class III swine hemorrhage model. Moreover, hydroxocobalamin had no apparent adverse effect on coagulation. The systemic vascular resistance may have been influenced by the nitric oxide scavenging properties of hydroxocobalamin, resulting in a decrease in cardiac output.

The authors thank Leeann Zarzabal, MS, for conducting the statistics and graph design.

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